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Effects of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) on Neutrophil Kinetics and Function in Normal Human Volunteers

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) (250 μg/m^2) was administered subcutaneously to 7 normal volunteers for up to 14 days to study its effects on neutrophil kinetics and function. With treatment, blood neutrophil counts rose gradually to peak at 3½ times baseline by day 14. At day 5 marrow mitotic cells were increased and post-mitotic cells decreased, and the transit time through the post-mitotic marrow pool accelerated (normal = 6.4 days, GM-CSF = 3.9 days; $P < 0.01$). Treatment had little effect on the blood neutrophil half-life (normal = 9.6 ± 1.3 hours; GM-CSF = 13.1 ± 2.4 hours, $P > 0.05$); or the neutrophil turnover rate (normal = 78.5 ± 11.9 × 10^7/cells/kg/day, GM-CSF = 91.4 ± 19.8 × 10^7/cells/kg/day, $P > 0.05$). GM-CSF reduced the number of neutrophils migrating to skin chambers (normal = 104 ± 25.0 × 10^6/cells, GM-CSF = 48.6 ± 16.0 × 10^6/cells; $P < 0.05$). Treatment increased expression of CD11b/CD18 but not Fcγ receptors (CD16, CD32, CD64). Treatment also stimulated the in vitro neutrophil respiratory burst in response to a variety of agonists, and this enhancement persisted for the duration of treatment. All subjects experienced local and systemic adverse effects and developed eosinophilia. This study indicates that GM-CSF at a dose of 250 μg/m^2 causes neutrophilia chiefly by accelerating delivery of neutrophils from the marrow to the blood and by decreasing migration from the blood to the tissues, with only a modest effect on neutrophil production and blood half-life. Am. J. Hematol. 57:7–15, 1998. © 1998 Wiley-Liss, Inc.

Key words: neutrophil kinetics and function; GM-CSF; blood half-life

INTRODUCTION

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein secreted by a variety of cell types, which supports the proliferation of macrophages and granulocytes in vitro and in vivo [1–3]. It was initially introduced into clinical practice as a stimulus for marrow recovery after autologous bone marrow transplantation for acute lymphocytic leukemia, non-Hodgkins lymphoma, and Hodgkins disease [4]. GM-CSF is also used to stimulate marrow recovery after chemotherapy, including chemotherapy for acute myelogenous leukemia [5,6]. Broader applications of GM-CSF, including its use for the treatment of infectious diseases, are currently under investigation [3,7–10].

In hematologically normal individuals, intravenous or subcutaneous administration of GM-CSF causes a rapid and reversible leukopenia followed by leukocytosis [11]. The acute leukopenia occurs within minutes and is attributed primarily to a transient increase in neutrophil adherence in the micro-vascular beds [12]. The leukocytosis that follows reflects the combined effects of GM-CSF in elevating blood levels of neutrophils, monocytes, and eosinophils [11,13] to increased leukocyte production in the bone marrow [14]. To clarify the mechanisms of the neutrophilia caused by GM-CSF and the effects of this cytokine on neutrophil and monocyte function, we have administered recombinant human GM-CSF (GM-

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CSF) to seven normal subjects for periods of up to 14 days.

MATERIALS AND METHODS

Human Subjects

Seven normal volunteers (3 women, 4 men; ages 21 to 32 years) were studied. All were nonsmokers on no medications without a recent history of significant illnesses. Each gave informed consent for this study, which was approved by the University of Washington Human Subjects Review Committee and Radiation Safety Committee.

Study Design: Overview

Each subject had a baseline medical history, physical examination, complete blood cell count, a skin chamber and oral wash for buccal neutrophils to measure in vivo neutrophil inflammatory responses. After these baseline studies, each subject received GM-CSF (250 $\mu g/m^2$/day subcutaneously) at 8:00 A.M. for 14 days. Daily blood cell counts were made immediately before the GM-CSF administration. After 5 days of GM-CSF, a bone marrow aspiration and repeat skin chamber and oral wash for buccal neutrophils were performed. On the following day blood neutrophil kinetic studies were performed, utilizing ^{3}H-diisopropylfluorophosphate (^{3}H-DFP). On the next day (day 7), subjects were injected with tritiated thymidine and blood samples drawn over the next 8 days for determination of the post-mitotic marrow neutrophil transit time. On days 0, 1, 5, and 12 neutrophil luminol-enhanced chemiluminescence in response to several stimuli and neutrophil and monocyte immunophenotypes were determined. Each study day, the subjects were seen by the investigator and/or the study nurse and any adverse events noted. At the end of the study, each subject completed a questionnaire regarding their general health and symptoms during the study period. These investigations paralleled previous studies of recombinant human granulocyte colony-stimulating factor (rhG-CSF) in normal subjects [15,16].

Special Reagents

For the in vivo studies, recombinant human GM-CSF (500 mcg/vial) (Sargramostim, as a lyophilized, yeast-expressed glycoprotein, Immunex Corporation, Seattle, WA) was reconstituted with sterile water prior to injection. For in vitro studies, recombinant human tumor necrosis factor (TNFα; specific activity 3.6×10^7 U/mg; endotoxin <0.06 endotoxin U/mL, Genentech, Inc., South San Francisco, CA) was used. Sources of other reagents were: Histopaque-1077, dextran, lipopolysaccharide (LPS; lyophilized powder, prepared using phenol extraction procedure from *Escherichia coli* 055:B5), phorbol myristate acetate (PMA), and luminol (Sigma Chemical Co., St. Louis, MO); formylmethionyl-leucyl-phenylalanine (FMLP) (Peninsula Laboratories, San Carlos, CA); RPMI 1640 was supplemented with HEPES buffer (10 mmol/L), (BioWhittaker, Inc., Walkersville, MD). The following murine monoclonal antibodies (mAbs) were obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA) for immunophenotype analysis: phycoerythrin-conjugated SK11 (Sk11-PE) (IgG_{2a}, anti-Leu-8 [anti-L-selectin]); D12-PE (IgG_{2a} anti-CD11b [antiCR3 β-chain, anti-LFA-1β]); and MϕP9-PE (IgG_{2b}, anti-CD14). Murine mAbs directed against Ig Fc receptors were purchased from Medarex, Inc. (Annandale, NJ): 3G8-FITC (IgG_1, anti-CD64 [anti-FcγRI]); IV.3-FITC (IgG_{2b}, anti-CD32 [anti-FcγRII]); and 32.2-FITC (IgG_1, anti-CD16 [anti-FcγRIII]). Irrelevant isotype-specific murine Ig fluorescence controls were also purchased from Becton Dickinson Immunocytometry Systems (San Jose, CA).

Blood Cell and Bone Marrow Examinations

Complete blood cell counts were performed utilizing an electronic particle counter (Coulter, model T540, Hialeah, FL). Differential counts on blood (100 cells) and bone marrow (500 cells) were performed on Wright's stained smears utilizing standard techniques. Results for marrow populations were expressed as a percent of the total nucleated cell population.

Neutrophil Transit Time

The marrow post-mitotic neutrophil transit time was measured following an injection of ^{3}H-thymidine (10 μCi/kg, 50–90 Ci/mmol, New England Nuclear, Boston, MA) on day 7. Morning blood samples were obtained on days 8 and 9 and every 12 h from days 10 through 15, as previously described [16]. Because of the substantial eosinophilia in the second week of GM-CSF treatment, neutrophil specific activity was determined serially for neutrophils separated from other leukocytes utilizing Ficoll-Hypaque separation, then CD16 microbeads (Miltenyi Biotec Inc., Auburn, CA), followed by NH_4Cl lysis of contaminating red cells [17]. The radioactivity of a sample of the isolated neutrophils was then determined by liquid scintillation counting. Cell specific activity was plotted as a function of time after injection of the isotope, with the resulting curve representing the sum of influx of labeled cells into the circulation from the marrow and the efflux of labeled cells into the tissues [18].

Blood Neutrophil Kinetic Studies

The blood recovery and survival of autologous neutrophils was measured on day 5, as previously described [19]. In brief, approximately 250 mL blood was withdrawn into a plastic bag containing citrate-phosphate-dextrose-adenine (CPDA1) anticoagulant (Fenwall PL130, Baxter Corporation, Deerfield, IL). The neutro-

phils were labeled in vitro with 200 μCi of ^{3}H-diisopropylfluorophosphate (DFP) (New England Nuclear, Boston, MA) for 40 min, and an aliquot was removed for determination of the specific activity of the infused neutrophils. The labeled cells were then reinfused over a 5–10-min period. Blood samples (20 mL) were obtained at 10 min and at 1, 2, 3, 4, 6, 8, 11, and 24 h after infusion for determination of neutrophil specific activity, isolating the cells by centrifugation over Ficoll-Hypaque followed by NH_4Cl lysis, as previously reported [19]. Blood neutrophil half-time was determined by the method of least squares through the most linear portion of a semilogarithmic plot of these data points, as previously described [17]. Neutrophil recovery was defined as the fraction of infused cells circulating at time 0, determined by the value of the y intercept of the extrapolated survival curve. The fraction of infused cells not recovered in the circulation was considered to represent the marginal pool. Neutrophil turnover was calculated as outlined by Athens et al. [20].

In Vivo Measurement of Inflammatory Response

For measurement of neutrophil migration to a site of cutaneous inflammation, a 2 cm^2 area of the volar forearm was abraded manually by scraping with a surgical scalpel blade, as previously described [21]. The site was covered with a glass chamber and fastened in place with adhesive compound and tape. The chamber was filled with a mixture of 10% autologous serum and saline and supplemented with 100 μ/mL streptokinase/streptodornase (Behringwerks AG, Marburg/Lahm, Germany) to prevent neutrophil clumping. The number of white cells in each fluid sample was determined with an electronic particle counter (Coulter, Hialeah, FL).

Neutrophil accumulation in the oral cavity was assessed by the method of Wright et al. [22]. The subject was asked to swish his/her mouth with 25 mL saline for 30 sec, return the specimen to a sputum cup, and to immediately repeat the procedure. These duplicate specimens were centrifuged at 200*g* for 10 min within 1 h of collection. The pellet was resuspended in 1 mL HBBS containing 2 μg/mL acridine orange and incubated 15 min in a 37°C shaking water bath. The cells were then resuspended and the number of neutrophils determined with a hemocytometer using fluorescence microscopy.

Immunophenotype Analysis of PMN and Monocytes

Cell surface expression of Leu8 (L-selectin), CD11b, CD18, CD14, CD16, CD32, and CD64 on neutrophils and monocytes was assayed in samples of erythrocyte-depleted whole blood by direct immunofluorescence flow cytometry using saturating concentrations of specific fluorescent-labeled murine mAbs. In brief, anticoagulated venous blood was collected from the normal human volunteers prior to (day 0) and just before GM-CSF administration (on days 1, 5, and 12). Following depletion of erythrocytes by hypotonic lysis in buffer (NH_4Cl, $NaHCO_3$, Na_2EDTA, pH 7.4), the cell preparation was resuspended in ice cold PBS at a concentration of 2×10^7 cells/mL, as previously described [23]. An aliquot of the erythrocyte-depleted whole blood suspension (50 μL, 10^6 cells) was added to 50 μL of mAb dissolved in PBS containing 0.1% bovine serum albumin and 0.1% sodium azide in wells of a 96-well vinyl microtiter assay plate (Costar 2596; Costar, Cambridge, MA) kept on ice. Cells were stained for 45 min at 4°C, washed once with PBS containing 0.1% sodium azide, then fixed with 1% paraformaldehyde in PBS. Gating on physical parameters was performed for separate analysis of PMN and monocytes, respectively. Simultaneous negative control staining reactions were performed with appropriate fluorescent-labeled irrelevant isotype-specific murine antibodies. The plates were kept at 4°C until the stained cells were analyzed by flow cytometry using a Coulter Elite (Coulter, Hileah, FL) set on logarithmic scale and Multiplus software (Phoenix Flow Systems, San Diego, CA). Mean fluorescence intensity (MFI) was calculated by subtraction of the mean fluorescence channel of the appropriate negative control.

Assay of PMN Luminol-Enhanced Chemiluminescence

PMNs were isolated from venous blood anticoagulated with 0.2% dipotassium ethylenediaminetetraacetic acid (K_2EDTA) by sequential sedimentation in Dextran T-500 (Pharmacia LKB Biotechnology, Piscataway, NJ) in 0.9% sodium chloride, centrifugation in histopaque-1077 (Sigma Chemical Co., St. Louis, MO), followed by hypotonic lysis of erythrocytes. The preparation contained greater than 97% polymorphonuclear leukocytes, of which greater than 95% were neutrophils. Cell viability was greater than 98% as determined by trypan blue exclusion.

Luminol-enhanced chemiluminescence was employed as a sensitive measure of the respiratory burst of human phagocytes as previously described [24,25]. Purified human neutrophils (1×10^6) were preincubated for 15 min in a 0.5 mL volume of RPMI 1640 with 10 mM HEPES and 15 μg/mL human serum albumin in polystyrene chemiluminescence cuvettes (Analytical Luminescence Laboratory, San Diego, CA) at room temperature. At the start of the assay, 10 μM luminol and the appropriate stimulus (100 nM PMA, 1 μM FMLP, 100 U/mL TNFα, or 1 μg/mL LPS), was added to the reaction mixture. Luminol-enhanced chemiluminescence was read for 10-sec intervals at the designated times with a Monolight 2001 luminometer (Analytical Luminescence Laboratory) set to integration mode. The assay was performed at room temperature. Chemiluminescence is reported as

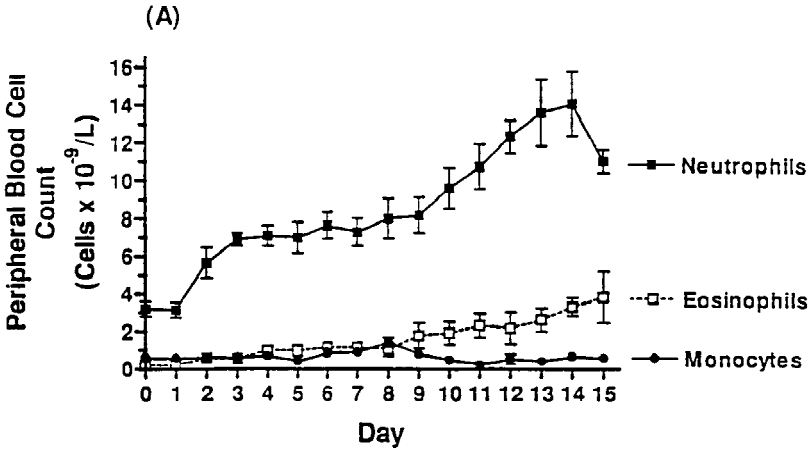

Fig. 1. Mean neutrophil, eosinophil, and monocyte counts for normal volunteers on GM-CSF. Counts were done before the daily injection which was given on Day 1 through Day 15.

relative light units/10^6 phagocytes/min (RLU/10^6 phagocytes/min).

Statistical Analysis

Results are expressed as mean ± 1 SEM unless otherwise specified. Student's *t*-test or Mann-Whitney test was used to determine the significance of differences between groups, as designated.

RESULTS

Clinical Observations

The daily injections of GM-CSF caused symptoms in all subjects. The most frequent were injection site reactions (itching and redness), bone pain and headache, as reported in other studies [2–8]. One male withdrew after 3 days because of fever, chills, headache, chest tightness, and bone pain.

Blood Cell Counts

Daily GM-CSF caused neutrophilia, a delayed eosinophilia, and a mild and transient monocytosis (Fig. 1). The time courses for these responses varied. The neutrophil count was significantly increased at 4 h after the first injection and thereafter the morning levels remained significantly elevated until treatment was discontinued. Eosinophil counts rose gradually from day 3 through day 15.

The platelet count decreased slightly during the course of the study. The lowest mean platelet count was 188 × 10^9/L ± 10 × 10^9/L (mean ± SE; n = 6) on day 13, compared to a baseline value day 0 of 212 × 10^9/L ± 14 × 10^9/L (Student's *t*-test, $P < 0.05$). The hematocrit and reticulocyte counts were not significantly affected by GM-CSF administration (data not shown).

Bone Marrow Examinations

Marrow aspirates on day 5 of GM-CSF treatment showed a statistically significant increase in the proportion of promyelocytes and myelocytes ($P < 0.05$, Mann-Whitney test) (Table I). There was a significant decrease in band and segmented neutrophils ($P < 0.05$, Mann-Whitney test). Marrow eosinophils were also significantly increased compared to normal ($P < 0.05$, Mann-Whitney test).

Neutrophil Emergence Time

Neutrophil emergence time measurements, which reflect the mean duration of maturation of cells of the neutrophilic lineage from the late myelocyte stage to the mature blood neutrophil, were decreased by GM-CSF treatment. On treatment the emergence time was 3.9 ± 0.3 (mean ± days SEM), compared to a normal of 6.4 ± 0.3 days (Student's *t*-test, $P < 0.01$), reflecting a 39% decrease in time the developing neutrophils spent in the marrow maturational compartment.

Blood Neutrophil Kinetic Studies

Neutrophils labeled with ^{3}H-DFP collected immediately before the daily injection of GM-CSF and reinfused just after the injection in 5 subjects circulated with a percent recovery of 86 ± 11% and a blood half-life of 13.1 ± 2.4 h. These values were not significantly different from normal values of recently studied normal subjects in our institution (normal recovery 65.2 ± 8.3%, half-life 9.6 ± 1.3 h) (Student's *t*-test, $P > 0.05$). The neutrophil turnover rate, a reflection of the degree of increased production stimulated by GM-CSF, was 91.4 ± 19.8 × 10^7 cells/kg/day, a value not significantly different from normal, 78.5 ± 11.9 × 10^7/cells/kg/day (Student's *t*-test, $P > 0.05$).

In Vivo Inflammatory Responses

Migration of neutrophils to skin chambers was significantly decreased by GM-CSF. For paired studies, the

TABLE I. Bone Marrow Differential Counts†

	Normal volunteers (n = 6)	Day 5 GM-CSF (n = 4)
Neutrophilic series		
Myeloblasts	0.6 ± 0.2	0.3 ± 0.0
Promyelocytes	2.8 ± 0.3	5.8 ± 1.1*
Myelocytes	9.7 ± 0.8	14.2 ± 1.3*
Metamyelocytes	11.3 ± 1.1	9.6 ± 0.4
Bands/stebs	13.2 ± 1.7	8.0 ± 1.1*
Segmented neutrophils	13.9 ± 1.7	6.1 ± 0.5*
Eosinophilia series	0.0 ± 0.0	8.2 ± 2.9*
Basophils/mast cells	0.1 ± 0.1	1.0 ± 0.5
Erythrocyte series	29.6 ± 2.5	34.9 ± 2.6
Lymphocytes	15.0 ± 1.3	11.7 ± 1.6

†Values represent X ± SE.
*$P < .05$ Mann-Whitney test (two-tailed).

mean cells per chamber decreased from 104.0 ± 25.0 × 10^6 to 48.6 ± 16.0 × 10^6, a decline of 50.5% ($P < 0.05$, Mann-Whitney test). Comparisons of the buccal neutrophil responses for day 0 vs. day 5 showed a decrease on GM-CSF in four out of five subjects, but the responses were quite variable and the differences were not significant for this small group of subjects (data not shown).

Immunophenotype Analysis

Immunophenotypic markers on neutrophils and monocytes were affected by GM-CSF treatment (see Fig. 2). For neutrophils, the expression of CD11b and CD18 increased on days 1, 5, and 12 (Student's *t*-test, $P < 0.05$); CD14 expression was increased on days 5 and 12 (Student's *t*-test, $P < 0.05$). In contrast, treatment reduced the surface expression of the low affinity IgG Fc receptor CD16 (Fcγ RIII) and had no effect on expression of CD32 (Fcγ RII) or CD64 (Fcγ RI). For monocytes, the greatest change was in expression of CD11b. CD14 on monocytes was increased on day 1 but significantly decreased by day 12 of GM-CSF treatment (Student's *t*-test, $P < 0.05$). As with the granulocyte population, CD16 expression was decreased by GM-CSF and expression of CD32 and CD64 was unchanged or decreased by this treatment.

Chemiluminescence

Blood neutrophils showed enhanced responses to PMA, FMLP, TNF, and LPS when tested for subjects on GM-CSF treatment (Fig. 3). The greatest effects were seen with the receptor-independent agonist PMA. When these responses were examined over time (Fig. 4), the peak rate of luminol-enhanced chemiluminescence responses for each of these stimuli was significantly greater on day 5 and day 12 than day 0 (pre-treatment) (Student's *t*-test, $P < 0.05$) and all were quantitatively greater on days 5 and 12 than day 1.

DISCUSSION

The colony-stimulating factors GM-CSF and G-CSF were introduced to clinical practice because of their capacity to stimulate neutrophil formation in vitro and in vivo with direct clinical application for accelerating marrow recovery after chemotherapy and bone marrow transplantation [1–3,11]. Over time it has been appreciated that these CSFs not only affect the production and kinetics of leukocytes but also their function, effects undoubtedly related to their clinical efficacy in the prevention and treatment of infections [26,27]. Much can be learned about these effects through studies in normal individuals. Because this study closely parallels our previous investigations of G-CSF in normal subjects [15,16], it provides some data for comparing these two agents.

GM-CSF administered once a day in a dose of 250 μg/m^2, a commonly used dose and schedule, stimulated neutrophilia by 4 h after injection, with a sustained increase in neutrophils for the entire treatment period. The gradual increase in blood eosinophils and the changes in marrow differential counts are similar to those previously reported [11,14,28]. At this dose, GM-CSF produced a neutrophilia comparable to that observed previously in normal subjects administered G-CSF at 30 μg/day (approximately 0.5 μg/kg subcutaneously) for a 14-day treatment period [15].

GM-CSF induces neutrophilia by accelerating the release of mature neutrophils from the marrow, from the compartment often referred to as the marrow neutrophil reserves [29]. This is reflected by the prompt increase in the circulating count within 4 h of GM-CSF administration, by the decreased proportion of mature marrow neutrophils compared to normal, and by the accelerated marrow transit time measured with ^{3}H-thymidine measured over study days 7 to 14. Acceleration of neutrophil maturation and a shortening of the transit time has been previously noted with other agents inducing neutrophilia, including endotoxin, etiocholanone (a testosterone metabolite), and G-CSF [16,29,30,31]. The degree of shortening with GM-CSF (normal 6.4 ± 0.6 days, GM-CSF 3.9 ± 0.5 days, 39% decrease) was intermediate between that observed for 30 μg/day of G-CSF (4.3 ± 0.7 days, 33% decrease) and 300 μg G-CSF (2.9 ± 0.4 days, 55% decrease). Other investigators have noted similar effects of GM-CSF [32].

Other factors also contribute to GM-CSF-induced neutrophilia. In the kinetic studies with DFP-labeled neutrophils, the percent recovery of labeled cells in the circulation was slightly increased (86 ± 11% for GM-CSF vs. 65 ± 8% for control; $P > 0.05$), suggesting, but not establishing, decreased margination as an effect of GM-CSF. The blood half-life was also modestly increased, (13.1 ± 2.4 h for GM-CSF vs. 9.6 ± 1.3 h for controls; *P*

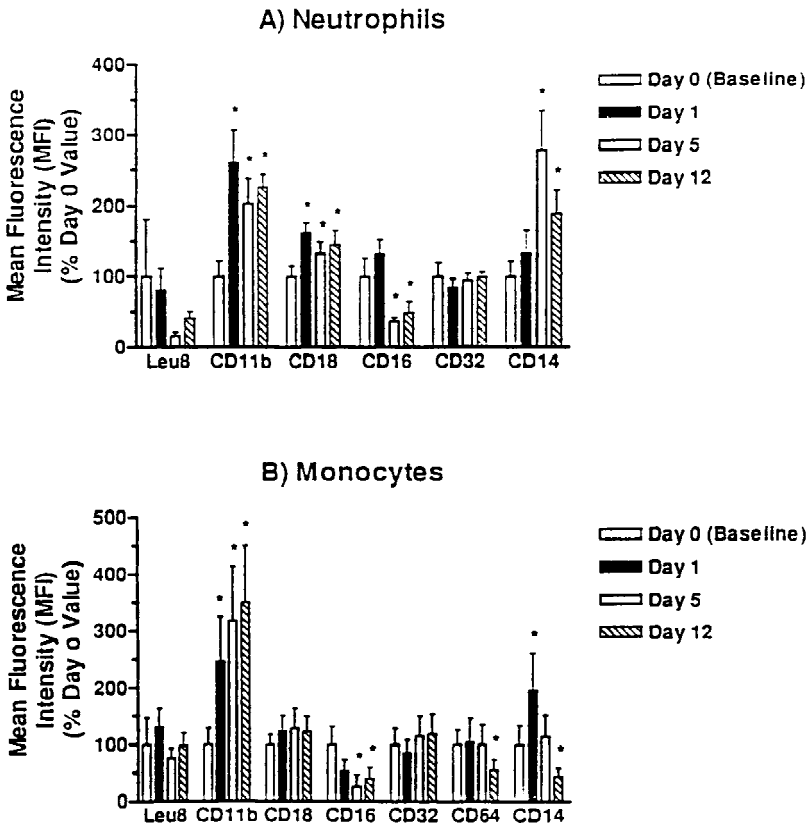

Fig. 2. Immunophenotype analysis of PMN and monocytes from normal human volunteers prior to and during daily administration of GM-CSF. Cell surface expression on (A) PMN and (B) monocytes was assayed by direct immunofluorescence flow cytometry of erythrocyte-depleted whole blood from normal human volunteers on Day 0 (pre-GM-CSF) and Days 1, 5, and 12 of GM-CSF administration. For each of the antigens examined, the data represent the relative intensity of specific fluorescence expressed as a percentage of the MFI present on Day 0. The results are reported as the mean ± SE from the six normal human volunteers. Asterisk (*) indicates a significant difference in MFI following administration of GM-CSF in vivo compared to Day 0 (control, pre-GM-CSF) ($P < .05$).

> 0.05), probably reflecting the early entry of the maturing neutrophils into the blood and prolonged survival due to reduced apoptosis [33,34]. The net effect was that with GM-CSF treatment the calculated neutrophil turnover rate was only slightly and not statistically greater than normal (91.4 ± 19.8 for GM-CSF vs. 78 ± 11.9 × 10^7 cells/kg/day for controls; $P > 0.05$). These results are comparable to those observed previously for 30 μg/day of G-CSF and are significantly less ($P < 0.01$) than for 300 μg/day of G-CSF (388.5 ± 141.2 × 10^7 cells/kg/day) [16]. Thus, GM-CSF at this dose appears to be a modest stimulus to neutrophil production, and the elevation in neutrophil counts can be attributed to several mechanisms [35].

In this study, GM-CSF significantly reduced neutrophil migration to a cutaneous abrasion, as measured by the skin chamber technique. Neutrophils in the chamber were reduced by approximately 50%, the same degree of reduction we noted previously in normal subjects administered 300 μg of G-CSF [16]. At present, the mechanisms for these effects are largely unknown. Reductions in neutrophil exudation with GM-CSF have been previously noted and attributed to alterations in expression of adhesion molecules [36,37]. In our studies of G-CSF, we noted that decreased migration occurred concomitant with reduced cell surface expression of CD11b and CD18 [38]. Because these proteins are essential for neutrophil adherence and migration in vivo, we presumed that the degree of expression of these proteins and the decrease in cell migration were related phenomena. In this study, however, GM-CSF induced significantly increased ($P < 0.05$) and sustained expression of these proteins throughout the treatment period, including the time point at which we measured decreased in vivo migration of neutrophils to the skin chambers. Thus, there is not necessarily a close correlation between quantitative expression of these surface markers and this measurement of the in vivo inflammatory response. We also observed that GM-CSF significantly increases monocyte CD11b but not CD18, and enhances, then significantly suppresses, monocyte expression of CD14. GM-CSF apparently does not increase the expression of Fcγ receptors on either neutrophils or monocytes; increased Fcγ receptors on neutrophils, however, have been noted after G-CSF by several investigators [27,39–41]. Others have also reported different effects of G-CSF and GM-CSF on surface expression of these proteins in short-term studies [42,43].

Mature neutrophils express surface receptors for GM-CSF and it is well established that in vitro exposure of neutrophils to GM-CSF primes them to an enhanced metabolic burst and chemiluminescence response in response to various stimuli [27,33,44,45]. In this study, we observed that this response is not maximal after a single

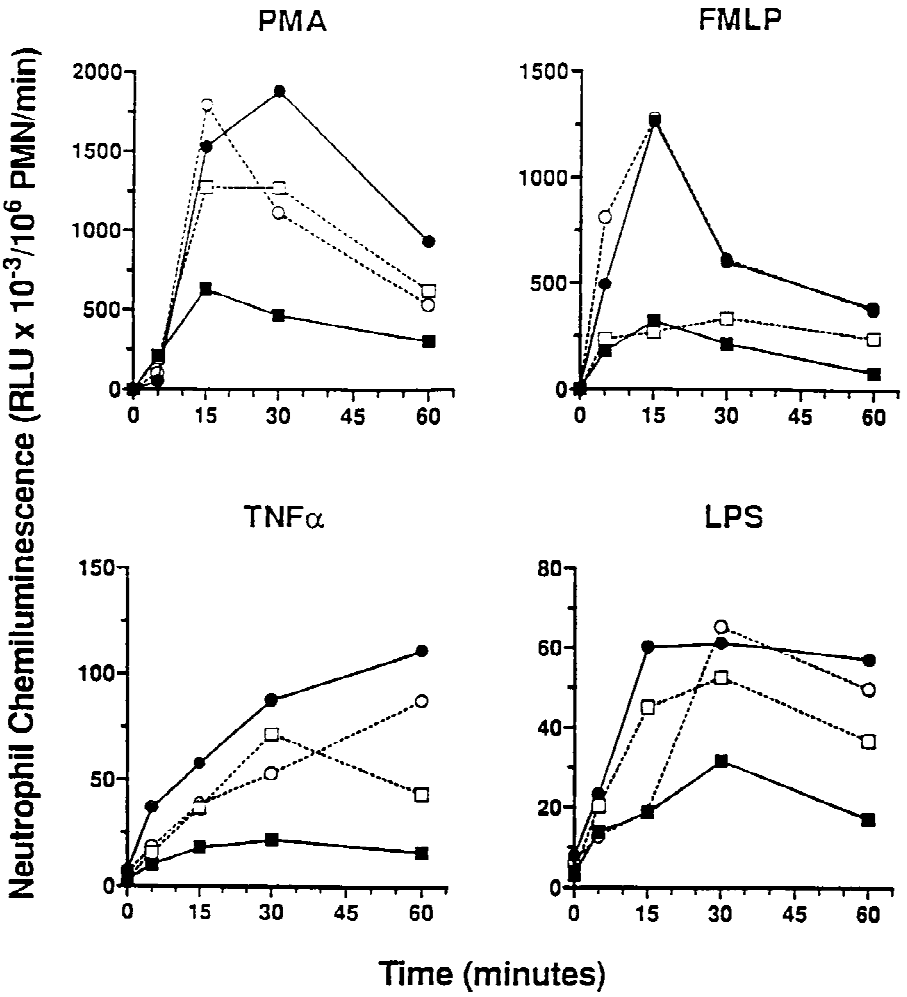

Fig. 3. Luminol-enhanced chemiluminescence of PMN from normal human volunteers prior to and during daily administration of GM-CSF in vivo. PMN in suspension were stimulated with PMA (100 nM), FMLP (1 μM), TNFα (100 U/mL), or LPS (1 μg/mL) as designated above. Conditions: (1) Day 0 (control, baseline pre-GM-CSF) (—■—); (2) Day 1 (--□--); Day 5 (—●—); and Day 12 (--○--). The data are reported as the rates of chemiluminescence observed at the designated time points (RLU/10⁶ PMN/min). The results represent the mean from the six normal human volunteers.

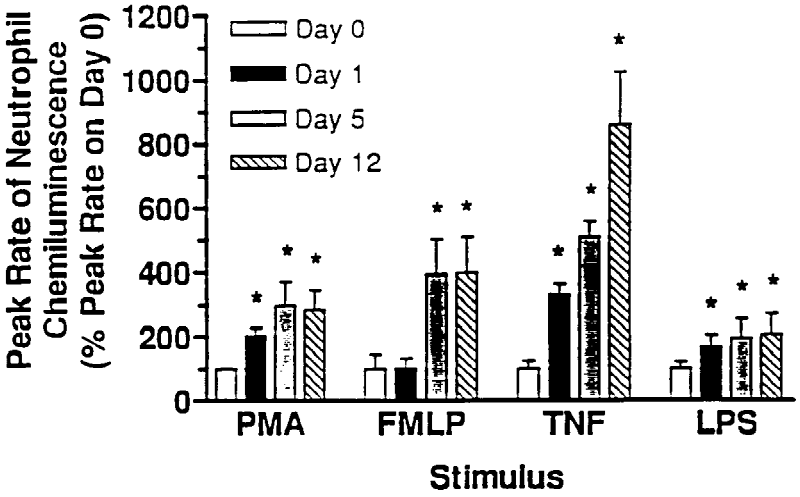

Fig. 4. Peak rates of luminol-enhanced chemiluminescence in PMN from normal human volunteers prior to and during daily administration of GM-CSF in vivo. PMN were isolated from normal human volunteers on Day 0 (pre-GM-CSF) and Days 1, 5, and 12 of GM-CSF administration and stimulated with PMA (100 nM), FMLP (1 μM), TNFα (100 U/mL), or LPS (1 μg/mL) as designated above. The data are reported as the peak rates of chemiluminescence observed with each stimulus. The results represent the mean ± SE from the six normal human volunteers. Asterisk (*) indicates a significant difference in chemiluminescence following administration of GM-CSF in vivo compared to Day 0 (control, pre-GM-CSF) ($P < .05$).

injection of GM-CSF but, rather, increases with continuation of treatment. The precise mechanism for this enhancement in neutrophils responsiveness over time and the clinical significance of these changes are not yet known.

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